REMARKS

Applicants hereby supplement their response to the final rejection, the complete remarks from which are hereby incorporated by reference.

New claim 17 is based on the subject matter claimed in claim 11 and supported at least at page 10, second paragraph of the specification. Thus, there is no new matter.

The present claims are directed to a very real situation when treating humans suffering from portal hypertension or related disease conditions (different from studies of rats undertaken by Garcia et al.) and by observing an "anti-portal hypertension effective dose".

The Examiner objected that the references cited in the last response were published after Garcia et al. and therefore could not be relied upon as to how the skilled person would understand Garcia et al.

Applicant respectfully submits that such objection is unjustified. There are equivalent or similar publications **prior** to Garcia et al. providing the same skilled person's information and understanding as submitted in the last response. This will be shown in the following discussion (see copies of cited references enclosed herewith). Indeed, the references and arguments presented hereinbelow provide further evidence for non-obviousness of the present invention.

J. Llach et al., *Gastroenterology* 94, 482-487 (1988) clearly demonstrated that a **low arterial blood pressure** (i.e. MAP) is associated with a **bad prognosis** in patients suffering from liver cirrhosis. Drugs which would reduce arterial pressure will worsen the prognosis. This issue is relevant and fully consistent with the submissions of our last response: Patients having liver cirrhosis and even having a very bad prognosis to survive by concurrently having low arterial pressure (i.e. MAP) should, when reading Garcia et al., not be treated in a way which would even **further decrease** MAP as reported by Garcia et al. It is noted that the dose showing a high drop in PVP had a **diminished persisted MAP** (cf. Garcia Abstract, lines 17-20 and 24-26). Noticeable PVP decrease thus is associated, according to Garcia, only in rats having such a drastic decrease of MAP that they never recovered and died. It is entirely wrong to

assume any therapeutic effect or implication from such disclosure in Garcia. It is further noted that the rats studied by **Garcia et al.** were **normal, non-cirrhotic rats** showing no portal hypertension *per se*. Note that Garcia merely studied hemodynamic effects of Sildenafil in the context of erectile dysfunction (see "Background"); Garcia never had a treatment of portal hypertension in mind. That is, in conjunction with other references such as Llach et al. it would have been be expected that the situation in cirrhotic organisms would be even worse. Thus, no therapeutic effect could have been expected, based on Garcia, to be associated with Sildenafil in connection with existing portal hypertension produced by liver cirrhosis.

The dilemma of a wrong treatment approach by merely trying to lower arterial pressure, in an attempt to thereby decrease PVP, is also explained in the present application (bottom of page 1 to first full paragraph on page 2 of the original English specification). Garcia et al. does not suggest that Sildenafil would indeed act differently from conventional vasodilating and arterial pressure reducing drugs like nitrates, beta-blockers, Vasopressin and the like, when (as found by the present invention) applied in humans in an appropriate dose to exert an anti-portal hypertension effect.

This dilemma – which, as noted, Garcia et al. does not resolve – is also demonstrated by the 1997 publication of **R.J. Groszmann**, *Gastroenterology* 113, 1795-1797 (1997).

Again, a similar understanding is presented by the 2002 publication of **Wiest and Groszmann**, *Hepatology* 35, 478-491 (2002) (ref. CD of the March 2006 IDS). As it is made unequivocally clear and, e.g., summarized in the section "Summary and Outlook" on pages 485-486 and illustrated in Fig. 5, an attempt to improve the situation in the splanchnic and systemic vasculature at the same time **worsens** the effects on the hypatic microvasculature. Note that Wiest and Groszmann are again closer to the critical situation of concern here by referring to the problems of portal hypertension, whereas Garcia, as noted, studies normal non-cirrhotic rats only. Reading Garcia et al. together with Wiest and Groszmann, again would **not** suggest to one of ordinary skill in the art to treat hypertension patients, because the MAP diminishing dose, which Garcia describes to be associated with a PVP drop, would appear as **deleterious and dangerous** in case of hypertension patients.

When considering Sildenafil in conjunction with liver cirrhosis, the publications of **Colle et al.** (see Abstract in *Digestive Disease Week* 2003; ref. CS in our March 2006 IDS) as well as their full paper published in *Liver International* 2004, 63-68, should be taken into account. According to Colle et al., **contrary to** Garcia et al., Sildenafil was reported to **increase PVP,** and this information is of more relevance as it is **referred to cirrhotic rats**. This, consistent with the prior understanding of the skilled person as explained above and in the last submission, would never have lead to an effective treatment in humans at an anti-portal hypertension effective dosage with the surprising and unexpected effects as demonstrated in the present application. See page 5, penultimate paragraph to page 6, first paragraph of the present English specification and the results of the Examples.

The above understanding is further confirmed by actual case reports shortly before the present priority date, relating to the use of Sildenafil. For example, **Tzathas et al.** reports in *Gastroenterol.* 97, 1856 (2002) about **Sildenafil** being a **risk factor** for acute variceal bleeding. This belief is contrary to the surprising findings of the present invention, by which it is demonstrated that when delivered at an appropriate anti-hypertension effective dose in humans, indeed a differential effect is exerted by PDE 5 inhibitors (see middle paragraph on page 8 of the original English specification and Examples of the application). It is noted that case reports similar to Tzathas et al. could be additionally cited.

It is again stressed that Garcia et al. studies **normal rats**. When an artificially high dosage of i.v. administration of Sildenafil led to an irreversible arterial pressure drop (i.e. the only dosage showing noticeable PVP decrease was eventually a lethal dosage), an even worse situation would have been expected by those skilled in the art for cases of **cirrhotic rats** as confirmed by Colle and others as explained above. At best, Colle et al. indicates Sildenafil as representing a drug in one line with other **general vasodilating agents**. Such agents, as explained above and as stated in the present application, prior to the present invention, were considered to be the wrong way of treating cirrhotic patients, especially patients having portal hypertension.

Moreover, Garcia et al. did not, and was not able to observe specific and differential effects of Sildenafil, which was recognized not before the present invention as causative for the

favorable effects when using appropriate dosage and administration to humans. Possibly, the specific effect was hidden by the extremely high and artificial i.v. dosage regimen in rats studied by Garcia et al. (as noted, the PVP decrease associated with a persisting diminished MAP cannot be seen as a specific effect; it was lethal). In any event, there is no suggestion in Garcia to skilled persons, much less to one of ordinary skill in the art, for the presently claimed invention.

In view of the discussion above, it is respectfully submitted that the present application is in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

If for any reason a fee is required, a fee paid is inadequate or credit is owed for any excess fee paid, the Commissioner is hereby authorized and requested to charge Deposit Account No. **04-1105**.

Dated: 2 Apr. '09

Respectfully submitted,

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post PVL/sham. SPP was not altered between sham, sham-indo and PVL-indo mice, but was increased after PVL in the absence of indomethacin treatment. In contrast, PSS was elevated in both PVL and PVL-indo when compared to sham and sham-indo groups. To delineate the isoform of cyclooxygenase involved, Cox-1 -/- and Cox-2 -/- mice were subjected to sham (n=4) or PVL (n=7) and after 7 days SPP, abdominal aortic flow, and PSS were evaluated. Contrary to the pharmacological blockade of Cox, the targeted deletion of the Cox-1 or Cox-2 isoforms in isolation was not able to abrogate the elevated hemodynamics observed with experimental PHT.

In conclusion, using a mouse model of prehepatic PHT the Cox inhibitor, indomethacin, prevented the development of PHT. However the use of targeted gene deletion of Cox-1 or Cox-2 failed to abrogate the hemodynamic changes. Thus, further investigations are required to identify which isoform is mediating this affect.

SPP and % PSS 7d post PVL or sham

	Splenic pulp pressure (cm/H_2O)	% portal systemic shunting
Wild type sham	6.9(0.42)	3.01(0.7)
Wild type PVL	13.8(0.6) *	55.7(12.6) *
PVL + Indomethacin	8.2(0.6)	68.7(14.4) *
Cox-1 -/- sham	6.2(0.9)	0
Cox-1 -/- PVL	9.5(2.3) *	66.1(15.6) *
Cox-2 -/- sham	6(0.8)	0.11(0.1)
Cox-2 -/- PVL	10.2(2) *	72.3(5.3) *

Values = Mean(SEM) n=(4-7) * = PVL/sham ttest p<0.05

S1553

Sildenafil in Rats with Cirrhosis and Portal Hypertension: Systemic and Splanchnic Haemodynamic Effects

Isabelle Colle, An De Vriese, Hans Van Vlierberghe, Norbert Lameire, Martine De Vos

OBJECTIVES: Sildenafil is a selective inhibitor of the cGMP-specific phosphodiesterase type V (PDE-V) in the corpus cavernosum. PDE-V is also present in the mesenteric artery. Cirrhosis is complicated by a splanchnic vasodilation attributed to a local overproduction of nitric oxide (NO). As sildenafil potentiates the effects of NO, it may further decrease mesenteric vascular tone and increase portal venous blood flow. The aim is to evaluate the effects of sildenafil on the systemic and splanchnic haemodynamics in an experimental model of cirrhosis. METHODS: Secondary biliary cirrhosis was induced in male Wistar rats by common bile duct ligation (CBDL, n = 8); control rats were sham-operated (sham, n = 7). Mean arterial pressure (MAP), portal venous pressure (PVP) and arterial mesenteric blood flow (MBF) were measured after intramesenteric (i.m.) (0.01 to 10 mg/kg) and after intravenous (i.v.) (0.01 to 10 mg/kg) administration of sildenafil. RESULTS: Baseline PVP was significantly higher in CBDL than in sham rats, whereas baseline MAP tended to be lower and MBF tended to be higher in CBDL compared with sham rats. Both i.m. and i.v. injection of sildenafil significantly decreased MAP and increased MBF and PVP in a dose-dependent way. The decrease in MAP was significantly lower in CBDL than in sham rats. The increase in MBF was significantly lower in CBDL than in sham rats. PVP tended to increase more importantly in sham rats than in CBDL. CONCLUSION: Sildenafil increases MBF and PVP and induces systemic hypotension. The effects are less pronounced in cirrhosis, suggesting vascular hyporesponsiveness to sildenafil. Although the rise in PVP in cirrhotic animals is smaller than in controls, it may present a risk for hemorrhagic complications. Further studies are necessary before prescribing sildenafil to patients with cirrhosis.

S1554

New Artificial Liver Support System Is Valuable Alternative to Liver Transplantation

Kazuaki Inoue, Makoto Yoshiba

Fulminant hepatic failure is a fatal syndrome accompanied by various metabolic disorders. Among these metabolic disorders, bleeding tendency due to impairment in synthesis of coagulation factors synthesis and hepatic coma due to impairment in detoxication of neuro-toxic substances are two major life threatening symptoms. Artificial liver support systems are expected to be effective for these two major symptoms. Plasma exchange has been most prevailing ALS method in Japan. Although PE is an effective method of replacement of depleted coagulation factors, its capacity in removal of toxic substances with a large pool is limited. To solve this limitation, we developed a new artificial liver support system consist of plasma exchange and hemodiafiltration using high performance membranes.54 patients with FHF admitted Showa University Fujigaoka Hospital from 1997 to 2002 underwent this ALS system. 18 cases were acute type and 36 cases were suacute type. Diagnosis criteria of FHF is a combination of prothrombin time less than or equal to 40% of control and hepatic coma greater than or equal to grade II. Acute type is defined as coma occurring within 10 days of the onset of first symptom and subacute type is defined as coma occurring later than 11 days. This ALS system consist of plasma exchange and hemodiafiltration. PE is performed by the membrane separation method using Plasmaflo (Asahi Medical co, Tokyo) with 40-60 units of fresh frozen plasma. HDF is performed with protein permeable synthesize high polymer membrane (BS-1.8 Torey Medical, Tokyo). Total amount of replacement fluid is 20-40l infused through postdilution route within 5-10 hours. Dialysis fluid flows concomitantly through HDF apparatus at a speed of 500ml/min. Replacement fluid and dialysis fluid contains bicarbonate buffer because a change from acetate to bicarbonate is impaired in FHF. Of 54 patients 51 (94.4%) fully regained their consciousness fully from their initial coma, and 38/54(70.4%)patients survived. Brain edema was observed only in two patients who fell into grade IV coma before admission. Hepatorenal syndrome was observed in two patients. This ALS system is highly effective and safe. The annual surveillance of Ministry of health labor and welfare 2001 reported that more than 80% of major hospitals in Japan adopted this ALS system and improved survival rate of acute type of FHF(62.7%). This ALS system combined with intensive medical treatment is valuable alternative to liver transplantation.

S1555

High Long-Term Survival and Function of Cryopreserved Primary Hepatocytes in Two Different Culture Systems

Meindert Sosef, Keishi Sugimachi, John Baust Jr., Mehmet Toner

The use of freshly isolated primary hepatocytes as the cell-source in bioartificial liver devices has shown benefits in several studies. For bioartificial livers to fully reach their clinical potential however, isolated hepatocytes need to be banked for extended periods with a minimum loss of survival and function. Accordingly, we evaluated the use of a cold-storage preservation solution, HypoThermosol (HTS), for the cryopreservation of primary hepatocytes, and assessed long-term post-thaw hepatocyte function in two different culture systems. Freshly isolated rat hepatocytes were frozen (-1°C/min to -80°C) in suspension in HTS supplemented with 10% (v/v) dimethyl sulfoxide (DMSO). After storage in liquid nitrogen, cells were rapidly thawed and maintained in a double collagen gel culture (monoculture, MC), or cocultured (CC) with 3T3-J2 murine fibroblasts. Serial measurements (up to 14 days) were made of albumin secretion, urea synthesis and responsiveness to stimulation with Interleukin-6 (IL-6) (MC). Survival of the cells was assessed by total intracellular LDH content (MC). Evaluation of survival and function of the cryopreserved samples was normalized to matched controls and reported as a percentage of control values ± standard deviation. Immediate post-thaw viability was 87% (± 4) in HTS-frozen samples, in comparison with control (100% (± 3)). Albumin secretion and urea synthesis stabilized following the first week of coculture and yielded 71% and 80% (MC) and 69% and 89% (CC) of function in the HTS group, respectively. Both groups showed identical morphological features of control and HTS-cryopreserved hepatocytes, and long term survival rates similar to that of their functional levels. Assessment of cellular response of fibrinogen production to cytokine (IL-6) challenge following cryopreservation revealed a similar pattern of transient upregulation in fibrinogen production and concurrent suppression of albumin secretion compared to non-frozen controls. Our results represent a significant advance in the successful cryopreservation of primary isolated hepatocytes that have been very difficult to preserve with high viability and long-term stable function in the past. These results may represent an important step forward to the utilization of cryopreserved hepatocytes in tissue engineering and regenerative medical applications such as in bioartificial liver devices.

S1556

Hepatic Coma in Post Graft Dysfunction can be Resolved by Albumin Dialysis (MARS)

Jan Stange, Deanna Oliver, Tarek Hassanein

Differential diagnosis of hepatic coma versus irreversible brain damage in post liver transplant graft dysfunction can be a challenging question in consideration of re-transplantation. To investigate the use of a liver support device (MARS) that has been shown to improve hepatic encephalopathy in cirrhosis with acute liver injury (Hepatology 2002; 36:949-958) in Post Graft Dysfunction (PGD) associated with hepatic coma was the purpose of this study. Methods: Patients analyzed had primary graft dysfunction without response to standard treatment and were assigned to MARS therapy. Data was analyzed from the international MARS Registry and UCSD Liver Center. Response to treatment, outcome and pre/post treatment biochemistry was analyzed. Results: 34 patients (16 females/19 males; mean age 50 +/-10) received median 4 (1-24) treatments of 6 hours each. 28% had severe hepatic encephalopathy, 20% had sepsis, 20% had renal failure. 22/35 (63%) patients survived, 4 out of them were retransplanted. 13/35 (37%) died, one of them after re-LTX. Bilirubin stabilized from 22.4 +/- 15.6 to 15.1 +/- 12 mg/dl (p<0.05), Creatinine from 2.3 +/- 0.7 to 1.65 +/- 0.8 (p<0.05). Hepatic encephalopathy improved from 2.8 +/- 1.5 to 1.6 +/-1.2 (p<0.005). In a living donation receipient with primary non function and suspected irreversible brain damage following cardiac arrest, the patient regained consciousness, justifying retransplantation. Conclusion: As in acute liver injury superimposed on cirrhosis, liver support can also resolve hepatic coma associated with post graft dysfunction.

S1557

The inhibitory effect of superoxides generated by Kupffer cells on liver regeneration

Heigo Takeuchi, Takanori Morikawa, Michiaki Unno, Masanori Suzuki, Seiki Matsuno

BACKGROUND & AIM: Since lipid peroxides were increased following partial hepatectomy and administration of antioxidants accelerate liver regeneration, it is likely that oxidative stress have inhibitory effects on restoration of liver mass. Recently it was reported that gadolinium chloride (GdCl3), known as a inhibitor of Kupffer cell, enhanced liver regeneration via upregulation of TNF-alpha and IL-6 following partial hepatectomy of rat. Since activated Kupffer cells can generate superoxides by NADPH oxidase, we elucidated the role of superoxides in liver regeneration. METHODS: Four groups of Male Sprague-Dawley rats were performed 70% hepatectomy. Group G were given GdCl3, group A were given NADPH oxidase inhibitor apocynin (4-hydroxy-3-methoxy-acetophenone), group AG were given both GdCl3 and apocynin, and group C were given vehicles only. Regenerated liver weight, BrdU labeling index, lipid peroxides, energy charge of the liver, serum level of TNF-alpha and IL-6 were measured. Liver regeneration were also tested using rats given superoxide dismutase(SOD) and transgenic mice manifesting excessive SOD.RESULTS: In group G, A and AG, liver weight were 20% increased compared group C on day 2 after hepatectomy. These 3 groups showed 20 to 40% increase of BrdU labeling index on day 1. Lipid peroxides were decreased to 30% in group G, A and AG compared to group C. In group C, energy charge of the liver was deteriorated to 70% of unresected liver on day 1, however, energy charge didn't show aggravation in group G, A and AG. In group G and AG, TNF-alpha and IL-6 were 2 times higher compared to group C on day 1, whereas those were unchanged in group A. Rats given intraperitoneal injection of SOD demonstrated accelerated restoration of liver weight and increase in BrdU labeling index whereas SOD transgenic mice failed to facilitate liver regeneration. CONCLUSION: These results indicate Kupffer cells are activated after hepatectomy and superoxides generated by Kupffer cells inhibit liver regeneration via

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Systemic and splanchnic haemodynamic effects of sildenafil in an *in vivo* animal model of cirrhosis support for a risk in cirrhotic patients

Colle I, De Vriese AS, Van Vlierberghe H, Lameire NH, DeVos M. Systemic and splanchnic haemodynamic effects of sildenafil in an *in vivo* animal model of cirrhosis support for a risk in cirrhotic patients.
Liver International 2004: 24: 63–68. © Blackwell Munksgaard, 2004.

Abstract: *Objectives:* Sildenafil is a selective inhibitor of the cGMP-specific phosphodiesterase type V (PDE-V) in the corpus cavernosum. PDE-V is also present in the mesenteric artery. Cirrhosis is complicated by a splanchnic vasodilation attributed to a local overproduction of nitric oxide (NO). As sildenafil potentiates the effects of NO, it may further decrease mesenteric vascular tone and increase portal venous blood flow. The aim is to evaluate the effects of sildenafil on the systemic and splanchnic haemodynamics in an experimental model of cirrhosis. *Methods:* Secondary biliary cirrhosis was induced in male Wistar rats by common bile duct ligation (CBDL, $n = 8$); control rats were sham-operated (sham, $n = 7$). The mean arterial pressure (MAP), portal venous pressure (PVP) and arterial mesenteric blood flow (MBF) were measured after intramesenteric (0.01–10 mg/kg) and after intravenous (i.v.) (0.01–10 mg/kg) administration of sildenafil.
Results: Baseline PVP was significantly higher in CBDL than in sham rats, whereas baseline MAP tended to be lower and MBF tended to be higher in CBDL compared with sham rats. Both intramesenteric and i.v. injection of sildenafil significantly decreased MAP and increased MBF and PVP in a dose-dependent way. The decrease in MAP was significantly less important in CBDL than in sham rats. The increase in MBF was importantly lower in CBDL than in sham rats. PVP tended to increase more significantly in sham rats than in CBDL. *Conclusion:* Sildenafil increases MBF and PVP and induces systemic hypotension. The effects are less pronounced in cirrhosis, suggesting vascular hyporesponsiveness to sildenafil. Although the rise in PVP in cirrhotic animals is smaller than in controls, it may present a risk for haemorrhagic complications. Further studies are necessary before prescribing sildenafil to patients with cirrhosis.

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Key words: sildenafil – cirrhosis – portal hypertension – splanchnic – haemodynamic

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The prevalence of impotence in patients with cirrhosis ranges from 25% to 60% (1–3). Sildenafil (Viagra®, Pfizer Ltd, Sandwich, Kent, UK) is a widely used and clinically effective drug for the treatment of erectile dysfunction (4, 5). After sexual stimulation, the non-adrenergic-non-cholinergic (NANC) nerves stimulate the corpora cavernosa, resulting in a release of nitric oxide (NO). In the vascular smooth muscle cells, NO stimulates the enzyme guanylate cyclase, thereby causing an enhanced production of cyclic guanosine monophosphate (cGMP). cGMP is responsible for relaxation of the smooth muscle cells of the corpora cavernosa, resulting in a filling of these corpora with blood and leading to erection (6). cGMP is degraded by a cGMP-specific phosphodiesterase, of which seven different isoforms exist (7). The most prominent isoform in the corpora cavernosa is type V (8). Sildenafil is a strong and selective inhibitor of the cGMP-specific phosphodiesterase type V (PDE-V), thus increasing the levels of cGMP in the corpora

cavernosa after sexual stimulation. The affinity of sildenafil for type V is 80–8500 times higher than for the other isoforms (9). Nevertheless, sildenafil in high doses can affect other phosphodiesterases, resulting in systemic side effects, such as a decrease in blood pressure (4, 10). Further, PDE-V is also present in the mesenteric artery of rats (11) and humans (12), in pulmonary arteries (13) and other vascular beds (14, 15), providing an additional potential for secondary effects.

Cirrhosis is characterised by an increased intrahepatic resistance and an important splanchnic vasodilation, both contributing to portal hypertension. The splanchnic vasodilatation has been attributed to a local overproduction of NO. As PDE-V is present in mesenteric arteries, it can be hypothesised that administration of sildenafil to cirrhotic patients may further decrease mesenteric vascular tone, resulting in a rise of portal venous blood flow and pressure.

If true, the use of sildenafil could be associated with an increased incidence of haemorrhagic complications in patients with cirrhosis and portal hypertension. A case of variceal bleeding after intake of sildenafil was recently reported in a patient with cirrhosis (16). There are no other data on the safety and potential side effects of sildenafil in patients with cirrhosis and portal hypertension.

Against this background, the present study evaluates the effects of sildenafil on systemic haemodynamics, portal pressure and arterial mesenteric blood flow in experimental animals with cirrhosis and portal hypertension.

Material and Methods

Induction of cirrhosis

The experiments were performed in 15 male Wistar rats (Iffa Credo, Brussels, Belgium) weighing 246 ± 2 g at the time of surgery. The animals received care in accordance to the national guidelines for animal protection and the protocols were approved by the Ethical Committee of experimental animals, Faculty of Medicine, University of Ghent, Belgium. In the first group of rats ($n = 8$), secondary biliary cirrhosis was induced by common bile duct ligation (CBDL), as previously described (17). In brief, under halothane (Fluothane®, Zeneca NV, Destelbergen, Belgium) inhalation anaesthesia, the common bile duct was exposed by median laparotomy and occluded by a double ligature with a non-resorbable suture (7-0 silk). The first ligature was made below the junction of the hepatic ducts and the second was made above the entrance of the pancreatic ducts. Before tying the upper part of the common bile duct, the duct was rinsed with 100 μl saline and 150 μl formalin 10% was injected. Formalin causes a sclerosing cholangitis, thus preventing the formation of a biloma (18, 19). The common bile duct was then resected between the two ligatures and the abdominal incision was closed. Haemodynamic studies were performed 4–5 weeks following bile duct ligation, as this delay is necessary for the development of secondary biliary cirrhosis (20).

A second group of animals ($n = 7$) was sham-operated (sham). Under halothane anaesthesia, the abdomen was opened by a mid-line incision, the liver and duodenum were manipulated and the abdomen was closed again. The animals were allowed free access to food and water.

Haemodynamic studies

All studies were performed in overnight-fasted rats. The animals were anaesthetised with thiobutabarbital (100 mg/kg intraperitoneally, Inactin®, RBI, Natick, MA, USA). The trachea was intubated and the jugular vein and carotid artery were cannulated for administration of drugs and continuous monitoring of the mean arterial blood pressure (MAP).

The femoral artery was cannulated and a small catheter was selectively advanced into the mesenteric artery. The mesenteric artery was exposed by a mid-line abdominal incision, and an ultrasonic blood flow sensor with an inner diameter of 0.6–0.8 mm was placed around the vessel, allowing continuous monitoring (Transonic Systems Inc., Ithaca, NY, USA) of the mesenteric artery blood flow (MBF). An ileocolic vein was cannulated with a 24-gauge catheter (Becton Dickinson, Insite W, Erembodegem-Aalst, Belgium), which was advanced into the portal vein and connected to a highly sensitive pressure transducer (Linearecorder F, WR 3701, Graphtec Corp, Yokohama, Japan). Portal venous pressure (PVP) was recorded with zero pressure assumed at the atrial level of the animal.

Sildenafil (Pfizer Ltd, Sandwich, Kent, UK) was administered intramesenterically in bolus at doses of 0.01, 0.1, 1 and 10 mg/kg body weight (BW). MAP, PVP and MBF were continuously recorded for 10 min after each dose, followed by a 10 min wash-out period. Thereafter, the catheter in the mesenteric artery was removed and sildenafil was administered intravenously (i.v.) in bolus at doses of 0.01, 0.1, 1 and 10 mg/kg BW. MAP, PVP and MBF were continuously recorded for 10 min after each dose followed by a 10 min wash-out period.

Histopathology of the liver

One sample of the liver was obtained in each experimental animal, fixed in 4% neutral buffered formalin and embedded in paraffin. Five micrometer sections were cut for light microscopical examination. The tissue was evaluated after a haematoxylin–eosin and a Picro Sirius Red staining F3B (Klinipath, Geel, Belgium). Sections were deparaffinised, rehydrated and stained briefly with Giemsa. Subsequently, sections were washed and stained with the Sirius Red solution, resulting in a brick red staining of all fibrillary collagen.

Statistical analysis

The results are expressed as mean ± standard error of the mean (SEM). The MBF response to sildenafil is expressed as the area under the curve (AUC) of the change in MBF (ml/min × min), as reported previously (21, 22). Analysis of variance, paired and unpaired t-tests were applied as appropriate. The results were considered statistically significant at a P level of <0.05.

Results

Characteristics of laboratory animals

Body weight was significantly lower in CBDL than in sham animals (Table 1). Plasma bilirubin, plasma aspartate aminotransferase levels and plasma alanine aminotransferase levels were significantly higher in CBDL than in sham rats (Table 1).

Baseline haemodynamic parameters

Baseline MAP was not significantly different between the experimental groups but tended to be higher in the sham rats (Table 1). Baseline MBF tended to be higher in CBDL compared with sham rats (Table 1). Baseline portal venous pressure (PVP) was significantly higher in CBDL than in sham rats (Table 1).

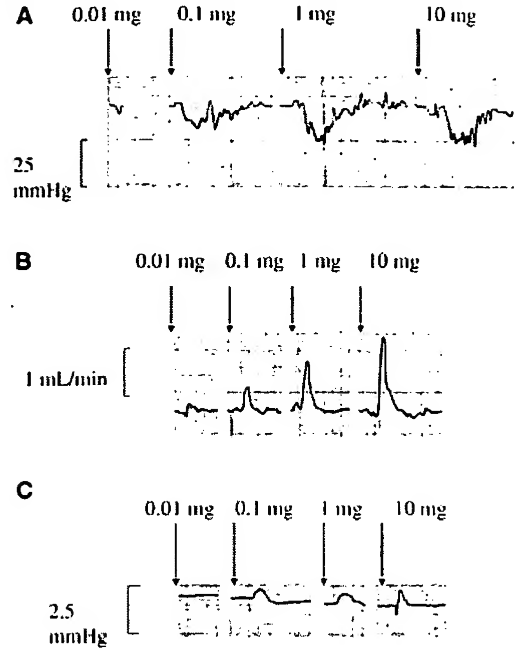

Fig. 1. A representative trace of the haemodynamic changes (A: MAP, B: MBF and C: PVP) after repetitive bolus of intramesenteric sildenafil in a cirrhotic rodent.

Haemodynamic parameters after sildenafil administration

A representative trace of the haemodynamic changes in MAP, MBF and PVP after incremental doses of sildenafil in a cirrhotic rodent is given in Fig. 1.

MAP

Both intramesenteric and i.v. sildenafil decreased MAP in all animals in a dose-dependent manner (Fig. 2A and B). The decrease in MAP was more important in the sham rats than in CBDL (Fig. 2B).

MBF

Intramesenteric and i.v. administration of sildenafil increased MBF in a dose-dependent manner (Fig. 3). The percentage increase of MBF was higher in sham rats than in CBDL (Fig. 3A and B).

Table 1. Characteristics and baseline haemodynamic parameters of the experimental animals

	CBDL	Sham	P
Body weight (g)	249 ± 6	291 ± 4	< 0.0001
Bilirubin (mg/dl)	8.3 ± 0.7	0.05 ± 0.01	< 0.0001
AST (IU/l)	317 ± 21	172 ± 6	0.0002
ALT (IU/l)	99 ± 4	70 ± 3	0.001
Baseline MAP (mmHg)	88 ± 3	97 ± 6	0.2
Baseline MBF (ml/min)	3.4 ± 0.2	3.2 ± 0.4	0.6
Baseline PVP (mmHg)	9.4 ± 0.6	6.6 ± 0.6	0.005

Body weight, plasma bilirubin levels, plasma aspartate aminotransferase (AST) levels and plasma alanine aminotransferase (ALT) levels in common bile duct ligated (CBDL) rats and sham-operated (sham) rats. Baseline mean arterial pressure (MAP), arterial mesenteric blood flow (MBF) and portal venous pressure (PVP) in the CBDL and sham rats.

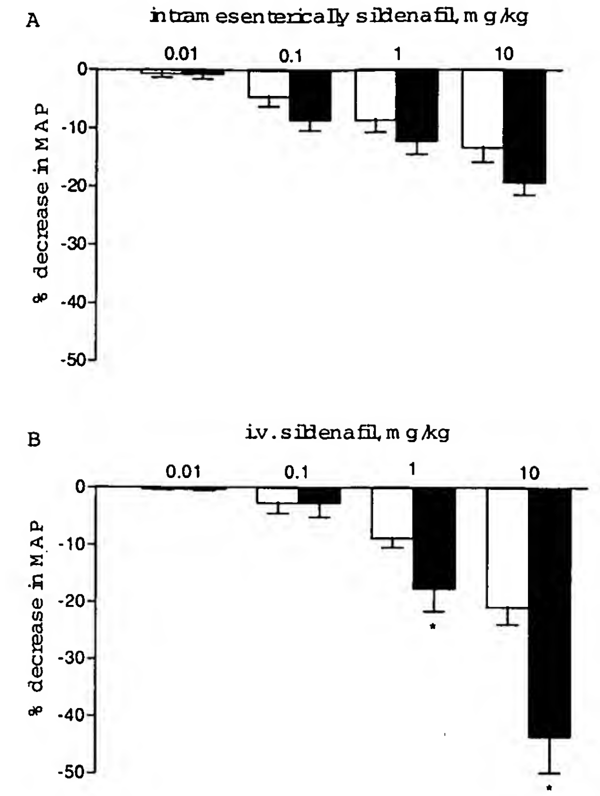

Fig. 2. Percent decrease in the mean arterial pressure (MAP) in common bile duct ligated rats ($n = 8$, open bars) and in sham rats ($n = 7$, full bars) for sildenafil given intramesenterically (0.01 to 10 mg/kg) (A) and given i.v. (0.01–10 mg/kg) (B). $^*P<0.05$ vs sham.

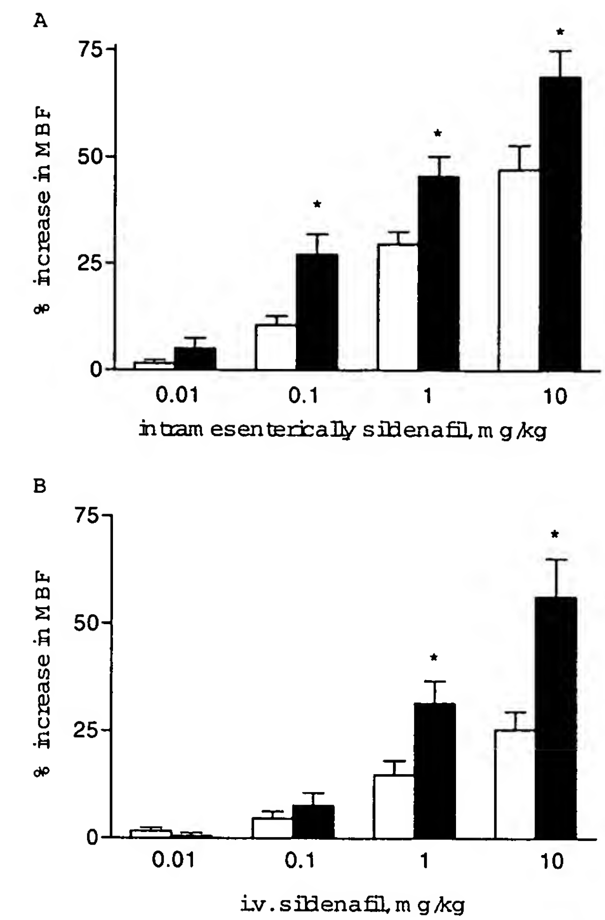

Fig. 3. Percent increase in arterial mesenteric blood flow (MBF) in common bile duct ligated rats ($n = 8$, open bars) and in sham rats ($n = 7$, full bars) for sildenafil given intramesenterically (0.01–10 mg/kg) (A) and given i.v. (0.01–10 mg/kg) (B). $^*P<0.05$ vs sham.

PVP measurements

Both intramesenteric and i.v. administration of sildenafil dose-dependently increased PVP in all animals (Fig. 4). The percentage increase in PVP tended to be higher in sham rats than in CBDL (Fig. 4A and B).

Histopathology of the liver

The liver of the CBDL animals was characterised by a marked ductular reaction and proliferation (Fig. 5A). Polymorphonuclear leucocytes infiltrated and surrounded some reactive periportal ductules, suggestive of cholangiolitis. On the Picro Sirius Red stain, periportal and porto-portal fibrosis and septum formation was evident (Fig. 5B). In some animals, early nodule formation is seen. However, this feature is not homogeneous: some parts of the liver have only periportal fibrosis. In sham animals, histological examination of the liver did not reveal any abnormalities.

Discussion

Cirrhosis is associated with an increased intrahepatic resistance and complicated by an important splanchnic vasodilation, both leading to portal hypertension and a hyperdynamic circulation. NO contributes to this splanchnic vasodilation by activating the production of cGMP, decreasing intracellular calcium and leading to relaxation of the vascular smooth muscle cells (23).

The present investigation is the first *in vivo* study demonstrating that sildenafil increases MBF and PVP in experimental animals with cirrhosis and in sham rats. In addition, sildenafil induced a pronounced and expected decrease in MAP in both animal groups (10, 24). The systemic and splanchnic haemodynamic responses to intramesenteric and i.v. sildenafil were similar.

The fall in MAP and the rise in MBF and in PVP were more pronounced in sham rats than in CBDL, suggesting hyporeactivity of the splanchnic and the systemic circulation to sildenafil in cirrhosis. The results are in accordance with previous data, demonstrating hyporeactivity to NO donors or other vasodilators in CBDL (25–33).

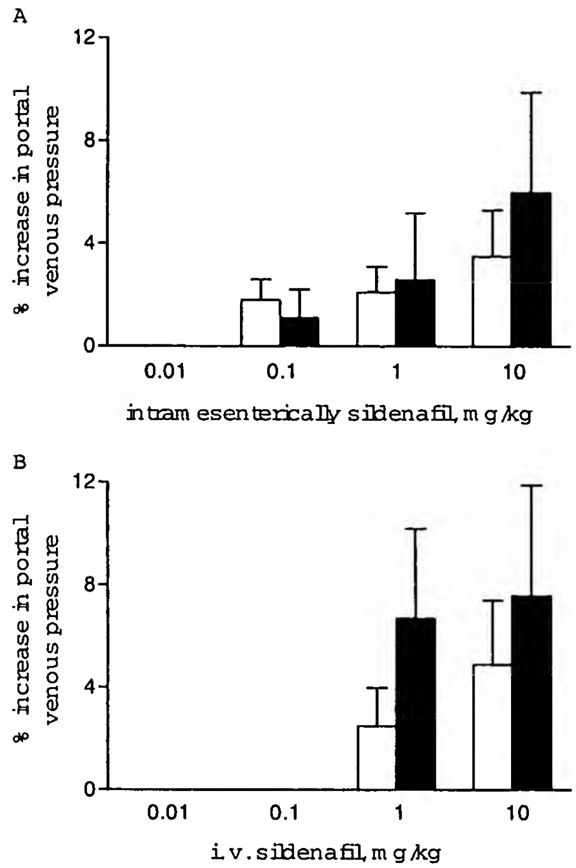

Fig. 4. Percent increase in portal venous pressure (PVP) in common bile duct ligated rats ($n = 8$, open bars) and in sham rats ($n = 7$, full bars) for sildenafil given intramesenterically (0.01–10 mg/kg) (A) and given i.v. (0.01–10 mg/kg) (B).

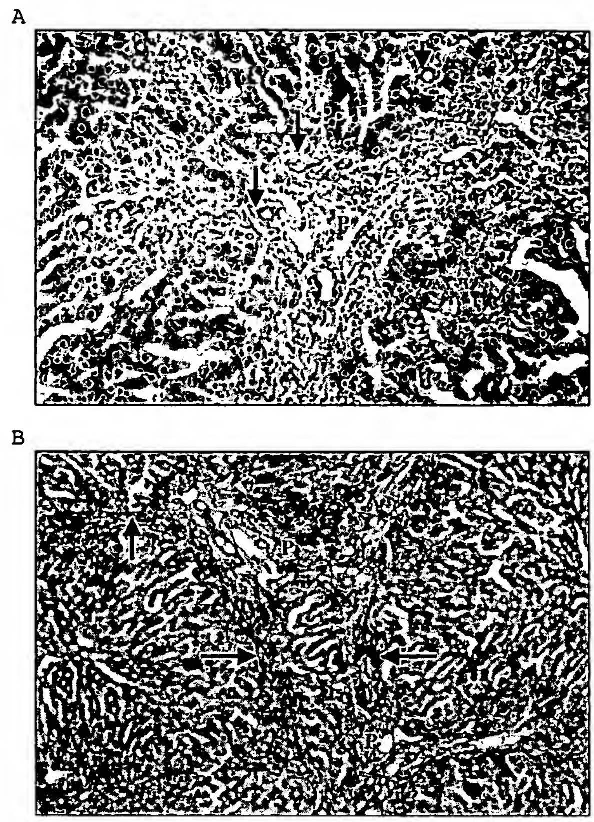

Fig. 5. Histopathology of the liver 4 weeks after common bile duct ligation and formalin 10% injection in experimental animals. (A) Haematoxylin–eosin staining (× 200 magnification). Portal tract (P); marked ductular reaction (arrow). (B) Sirius Red staining (× 100 magnification). Portal tract (P); marked ductular reaction; periportal and porto-portal fibrosis (arrows).

The present results may have important clinical implications in patients with cirrhosis.

First, sildenafil increases MBF and PVP in cirrhotic animals. Although less pronounced than in controls, a sudden increase in PVP in cirrhosis can lead to rupture of oesophageal varices. So far, one case of a rupture of oesophageal varices four hours after the intake of 25 mg sildenafil was reported in a cirrhotic patient who apparently was at low risk for oesophageal bleeding (16).

Further, sildenafil results in hypotension, which may be deleterious in patients with cirrhosis who already have a low blood pressure. In addition, the observed hyporeactivity of the splanchnic and systemic circulation to sildenafil suggests the presence of hyporeactivity in the corpora cavernosa as well. The resultant diminished efficacy of sildenafil could perhaps lead to overuse and automedication of the patient.

Finally, as the plasma clearance of sildenafil is delayed in patients with cirrhosis, the above-described haemodynamic actions of sildenafil may increase the potential for side effects in these patients (34).

In conclusion, sildenafil increases mesenteric blood flow and portal venous pressure and decreases blood pressure in experimental animals with cirrhosis. The effects are less pronounced than in control animals, suggesting mesenteric and systemic hyporeactivity to sildenafil. These side effects may cause haemorrhagic complications in patients with cirrhosis, especially in view of the prolonged half-life and the expected lower efficacy, which may motivate the patient to increase the dose. Clinical haemodynamic investigations in human cirrhosis should be performed before sildenafil can be safely prescribed to these patients.

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GASTROENTEROLOGY 1988;94:482–7

Prognostic Value of Arterial Pressure, Endogenous Vasoactive Systems, and Renal Function in Cirrhotic Patients Admitted to the Hospital for the Treatment of Ascites

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To identify prognostic factors in cirrhotic patients admitted to the hospital for the treatment of an episode of ascites, a survival analysis was performed in a series of 139 patients hospitalized in our Unit between 1980 and 1985. Mean follow-up was 12.8 ± 14.2 mo (mean ± SD). A total of 38 variables based on history, physical examination, hepatic biochemical tests, renal function tests, and endogenous vasoactive systems were analyzed for prognostic value. Eighteen of these variables had prognostic value in the univariate analysis. A multivariate analysis (Cox's regression method) disclosed that 7 of these 18 variables had independent prognostic value. Of these independent predictors of survival, mean arterial pressure and plasma norepinephrine concentration were the variables that best predicted prognosis. Two other variables that independently correlated with survival were urinary sodium excretion and glomerular filtration rate. The remaining three independent predictors of survival were nutritional status, hepatomegaly, and serum albumin concentration. Therefore, these findings indicate that, in patients with cirrhosis and ascites, parameters estimating systemic hemodynamics and renal function are better predictors of survival than those routinely used to estimate hepatic function.

The development of sodium and water retention and the formation of ascites in cirrhosis are related to a systemic hemodynamic disturbance that leads to effective hypovolemia, arterial hypotension, overactivity of the renin-angiotensin and sympathetic nervous systems, and nonosmotic hypersecretion of antidiuretic hormone (1–5). The stimulation of these endogenous vasoconstrictor systems may also be involved in the pathogenesis of functional renal failure in these patients (6,7). Renal function abnormalities and the increased activity of endogenous vasoactive systems are of great interest in cirrhosis not only because they are involved in the pathogenesis of ascites, but also because they may be of value in making prognoses. Functional renal failure is a well-recognized prognostic marker in patients with cirrhosis and ascites (8–10). On the other hand, we have shown that sodium excretion and plasma renin activity are also of prognostic significance in these patients (11). These latter findings have recently been confirmed (12).

During the last few years we carefully evaluated renal function, arterial pressure, and activity of endogenous vasoactive systems in a large number of patients with cirrhosis hospitalized for the treatment of ascites following an identical protocol. These measurements were performed as part of several studies of the pathophysiological and therapeutic aspects of ascites and renal failure in cirrhosis (13–16). As the majority of these patients have been closely followed up by us during the course of the disease, they constitute a unique population to investigate the prognostic value of these parameters in cirrhosis. In the current study we report the results of a survival analysis of these subjects.

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Patients and Methods

The present study includes 139 unselected patients admitted to our Unit between May 1980 and August 1985 for the treatment of an episode of tense ascites. We did not include patients with tense ascites presenting with gastrointestinal bleeding within 1 mo before the study, hepatic encephalopathy, bacterial infection, severe renal failure (serum creatinine >3 mg/dl), echographic data suggesting hepatoma, or respiratory or cardiac failure. In 117 patients there was histologic confirmation of cirrhosis at the time of the analysis of the results (January 1986). Written informed consent was obtained from each patient studied. The protocol was approved by the Investigation and Ethics Committee of the Hospital Clínic i Provincial of Barcelona.

A detailed account of the history and physical examination was recorded on admission. A diagnostic paracentesis was performed in all patients, and the protein concentration of ascitic fluid was measured. After a minimum of 5 days on a low-sodium diet (40 mEq/day) and no diuretic treatment, a 24-h urine sample was collected to measure urine osmolality and urinary sodium and potassium excretion. The day after, blood samples were taken to measure plasma renin activity, plasma norepinephrine and antidiuretic hormone concentration, hepatic biochemical tests, and renal function tests. These samples were obtained after overnight fasting from solid food and following 2 h of bed rest. At this time, three measurements of arterial pressure were taken at 10-min intervals by sphygmomanometry. Subsequently, the inulin clearance was performed to measure the glomerular filtration rate. Methods used for these studies have been described in detail elsewhere (17). After completion of these studies, patients were treated either with diuretics (spironolactone or spironolactone and furosemide; 105 cases) or with paracentesis (4–6 L/day) and intravenous infusion of albumin (40 g/day) until the disappearance of ascites (34 cases). No other drug was given unless it was clearly needed. In 10 patients not responding to diuretics, a peritoneovenous shunt was inserted at some time during the course of the illness. Twenty of the 139 patients died during the hospitalization period, during which renal function was studied (25.6 ± 18.1 days after the study; range, 4–72 days). One-hundred three of the remaining 119 patients were followed closely throughout the illness by staff members of our Unit. In 10 other patients we learned by telephone or mail whether they were alive or, if they were not, the date of death. Finally, 6 patients were lost to follow-up, 5 of them discharged from the hospital 10, 12, 20, 23, and 55 days after the study, respectively, and one at 23 mo. In the whole series the mean follow-up period was 12.8 ± 14.2 mo.

Table 1. Variables With Prognostic Value in Patients With Cirrhosis and Ascites

Previous episodes of ascites	Blood urea nitrogen
Previous episodes of encephalopathy	Serum sodium
Hepatomegaly	Urinary sodium excretion
Nutritional status	Urinary potassium excretion
Serum bilirubin	Urine osmolality
Serum albumin	Plasma renin activity
Serum γ-globulin	Plasma norepinephrine
Glomerular filtration rate	Mean arterial pressure
Serum creatinine	Protein concentration in ascitic fluid

Table 2. Variables Without Prognostic Value in Patients With Cirrhosis and Ascites

Age	γ-Glutamyl transpeptidase
Sex	Prothrombin activity
Etiology (alcoholic or nonalcoholic)	Serum cholesterol
Previous episodes of gastrointestinal bleeding	Platelet count
Splenomegaly	Leukocyte count
Hepatic stigmata	Urine volume
Peripheral edema	Serum potassium
Aspartate aminotransferase	Plasma osmolality
Alanine aminotransferase	Plasma antidiuretic hormone concentration
Alkaline phosphatase	Type of treatment (diuretics or paracentesis)

Statistical Analysis

A total of 38 variables (Tables 1 and 2) were analyzed as possible predictors of survival. Univariate analysis of survival was carried out by computing survival curves according to the Kaplan and Meier method (18). Curves were statistically compared using the Mantel–Cox test. In assessing the prognostic value of arterial pressure, mean arterial pressure [diastolic pressure + 1/3(systolic pressure − diastolic pressure)] was used. Nutritional status was assessed by physical examination and graded as "excellent," "good," or "poor." The presence of hepatomegaly and splenomegaly could not be assessed in 6 patients with tense ascites who died without responding to therapy. In addition, in 9 other patients one of the following variables was not recorded: mean arterial pressure (3 patients), plasma norepinephrine (2 patients), nutritional status (2 patients), previous encephalopathy (1 patient), and protein concentration in ascitic fluid (1 patient). Variables that achieved statistical significance ($p < 0.05$) in the univariate analysis were included in a multivariate analysis using the stepwise Cox regression procedure (19) to obtain those variables that independently correlated with survival. All these calculations were made with the BMDP (1L and 2L) program (20). Data are presented as mean ± standard deviation.

Results

Clinical Characteristics of the Patients at Inclusion

Of the 139 patients studied, 86 were men and 53 women. The mean age was 55.3 ± 11.6 yr (range 26–82 yr). Eighty-nine patients (64%) were considered to have alcoholic cirrhosis (constant ethanol intake >80 g/day) (21), 41 (29%) cryptogenic cirrhosis, and 9 (6%) hepatitis B surface antigen-associated cirrhosis. Most patients had advanced liver disease

as indicated by the high frequency of positive history of complications (i.e., ascites, encephalopathy, gastrointestinal hemorrhage) and a marked alteration of liver function tests. One hundred four patients (75%) had had at least one previous episode of ascites, 35 (25%) had had an episode of encephalopathy, and 34 (24%) of gastrointestinal bleeding. Serum bilirubin levels (mean 2.7 ± 2.7 mg/dl, range 0.3–20 mg/dl) were increased (>1 mg/dl) in 104 patients, and serum albumin concentration (mean 27.5 ± 4.8 g/L, range 13–39 g/L) and prothrombin activity (mean 61% ± 16%, range 18–100) were reduced (<35 g/L and 80%, respectively) in 129 and 117 patients, respectively.

On physical examination, 81 patients (58%) were found to have hepatomegaly, 71 patients (51%) splenomegaly, 120 patients (86%) hepatic stigmata, and 99 patients (71%) peripheral edema. A poor nutritional status was observed in 28 patients (20%).

Twenty-seven patients (19%) had renal failure as defined by a glomerular filtration rate lower than 50 ml/min. The mean values of the glomerular filtration rate, blood urea nitrogen, and serum creatinine concentration in these 27 patients were as follows: 31 ± 12 ml/min, range 15–48 ml/min; 45 ± 15 mg/dl, range 28–93 mg/dl; and 2.1 ± 0.6 mg/dl, range 1.6–3 mg/dl, respectively. Twenty-three of these patients fulfilled the criteria of functional renal failure (oliguria, urine-to-plasma osmolality ratio higher than unity, urine sodium concentration <10 mEq/L, normal fresh urine sediment, and no proteinuria). In 5 of these patients renal failure followed a rapidly progressive course and they died within a very short period of time (7, 10, 11, 27, and 31 days after the study, respectively). The remaining 18 patients had a steady functional renal failure (22). In 4 patients renal failure was associated with hematuria or proteinuria, or both, suggesting a glomerular disease. This was confirmed by renal biopsy in 2 cases.

The mean serum sodium concentration in the whole series was 132.7 ± 5.7 mEq/L (range 114–145 mEq/L). Thirty-two patients had hyponatremia at admission (serum sodium < 130 mEq/L). Most patients exhibited avid sodium retention as indicated by a very low urinary sodium excretion (mean value in the whole series 6.8 ± 12.2 mEq/day, range 0.1–80 mEq/day. Plasma renin activity and plasma norepinephrine and antidiuretic hormone concentrations were increased in a great number of patients. The mean values in the whole series were as follows: plasma renin activity 8.7 ± 11.0 ng/ml · h, range 0.1–68.5; norepinephrine 700 ± 522 pg/ml, range 66–3521; and antidiuretic hormone concentration 4.2 ± 1.7 pg/ml, range 0.6–9.8 (normal values in our laboratory: 1.3 ± 1.0 ng/ml · h, 275 ± 184 pg/ml, and 2.4 ± 0.8 pg/ml, respectively). Mean arterial pressure was ≤80 mmHg in 64 patients (mean value in the whole series 83 ± 10 mmHg, range 60–123 mmHg). Protein concentration in ascitic fluid was ≤1 g/dl in 84 patients. The mean protein concentration in ascitic fluid in the remaining 54 patients was 1.9 ± 0.9 g/dl (range 1.1–4.1 g/dl).

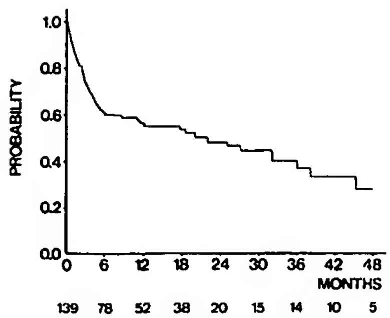

Figure 1. Probability of survival in all patients included in the study. Figures under the abscissa represent the number of patients at risk at any given period.

Survival

At the time of analysis of the results 65 patients (47%) were alive, 68 (49%) had died, and 6 (4%) were lost to follow-up. The probability of survival in the whole series was 62%, 56%, and 49% at 6, 12, and 24 mo, respectively (Figure 1). The causes of death were liver failure in 33 patients (48%), gastrointestinal bleeding in 17 (25%), bacterial infection in 12 (18%), and cerebrovascular accident and aspiration pneumonia in 1 patient. The causes of death in 4 patients were unknown. Patients who died of liver failure did not differ from those who died of gastrointestinal bleeding in any of the variables studied.

Factors Correlating With Survival

Eighteen of the 38 variables studied were found to be of value in making a prognosis in the univariate analysis (Table 1). The remaining 20 variables without prognostic significance are shown in Table 2. The following are variables from history, physical examination, and hepatic biochemical tests associated with a poor prognosis: previous episodes of ascites ($p = 0.04$), previous episodes of encephalopathy ($p = 0.04$), absence of hepatomegaly ($p = 0.026$), poor nutritional status ($p = 0.0001$), serum bilirubin >2 mg/dl ($p = 0.0025$), serum albumin <28 g/L ($p = 0.01$), and serum γ-globulin >23 g/L ($p = 0.048$). Variables related to renal function associated with a poor prognosis were glomerular filtration rate <50 ml/min ($p = 0.01$), serum creatinine >1.2 mg/dl

Table 3. Variables With Independent Prognostic Value in the Multivariate Analysis

Variable	p
1. Mean arterial pressure	0.0001
2. Plasma norepinephrine	0.0001
3. Nutritional status	0.004
4. Hepatomegaly	0.007
5. Serum albumin	0.016
6. Urinary sodium excretion	0.002
7. Glomerular filtration rate	0.04

(p = 0.04), blood urea nitrogen >30 mg/dl (p = 0.04), serum sodium concentration <133 mEq/L (p = 0.004), urinary sodium excretion <1.5 mEq/day (p = 0.0011), urinary potassium excretion <22 mEq/day (p = 0.019), and urine osmolality >530 mosmol/kg (p = 0.045). Other variables that correlated with a shortened survival were plasma renin activity >4.5 ng/ml · h (p = 0.0001), plasma norepinephrine concentration >570 pg/ml (p = 0.0012), mean arterial pressure ≤82 mmHg (p = 0.00001), and protein concentration in ascitic fluid ≤1 g/dl (p = 0.015).

Of these 18 variables with prognostic significance in the univariate analysis only seven were found to have an independent prognostic value in the multivariate analysis: mean arterial pressure, plasma norepinephrine concentration, nutritional status, hepatomegaly, serum albumin concentration, urinary sodium excretion, and glomerular filtration rate (Table 3). Figures 2 and 3 show the probability of

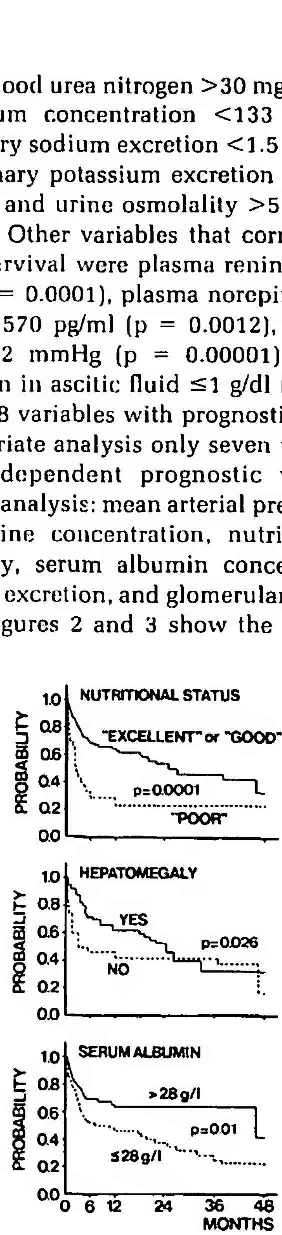

Figure 2. Probability of survival in all patients included in the study classified according to the nutritional status, presence or absence of hepatomegaly, and serum albumin concentration.

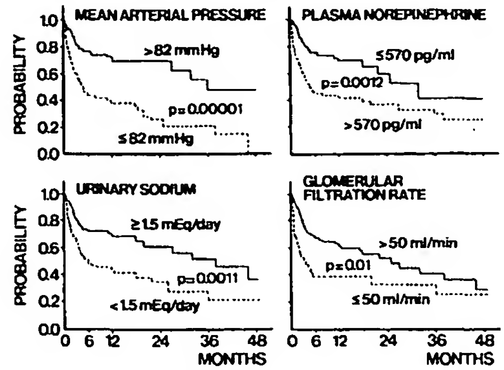

Figure 3. Probability of survival in all patients included in the study classified according to mean arterial pressure, plasma norepinephrine concentration, urinary sodium excretion, and glomerular filtration rate.

survival in all patients studied classified according to the variables with independent prognostic value.

Discussion

The results of the current study show that many data from history, physical examination, and hepatic biochemical tests are of prognostic significance in cases of cirrhosis with ascites.

Several parameters that reflect the degree of hepatic insufficiency such as previous episodes of ascites and hepatic encephalopathy, poor nutritional status, hyperbilirubinemia, and hypoalbuminemia were associated with a poor prognosis. These findings are in agreement with previous investigations in patients with cirrhosis (23–25). In addition, our results confirm that serum γ-globulin concentration is a prognostic indicator in these patients (23,25). Hypergammaglobulinemia in cirrhosis is a consequence of an increased antibody production caused by passage of intestinal antigens to the general circulation (26). As this latter abnormality is probably related to the intrahepatic shunting of blood (27), serum γ-globulin concentration probably reflects the degree of alteration in hepatic microcirculation. Several studies have shown that liver volume correlates with hepatic perfusion (28,29). This may explain the prognostic value of liver size. Liver size was also found to be of prognostic significance in the study of the Copenhagen Study Group for Liver Diseases, which included 488 patients (23).

An outstanding observation of the present study was that patients with protein concentration in ascitic fluid ≤1 g/dl showed a significantly shorter survival than those with an ascites protein concentration >1 g/dl. Recently, Runyon et al. (30) have shown that ascites protein concentration correlates closely with the antibacterial activity of ascitic fluid.

Furthermore, it has been observed that cirrhotic patients with protein concentration in ascitic fluid ≤1 g/dl are predisposed to developing spontaneous bacterial peritonitis (31). This may partially account for the prognostic significance of this parameter in our patients. In fact, 15 of the 84 patients (18%) with low protein concentration in ascitic fluid included in the current study developed spontaneous bacterial peritonitis during follow-up, and 8 of these patients died with this complication. In contrast, none of the 54 patients with protein concentration in ascitic fluid >1 g/dl developed spontaneous bacterial peritonitis during follow-up. On the other hand, it has been reported that in patients with cirrhosis portal pressure correlates inversely with ascitic fluid total protein concentration (32). This may also account for the prognostic value of ascites protein concentration in these patients.

Numerous parameters of renal function were also found to be of value in predicting prognosis in the univariate analysis. As expected, measurements estimating glomerular filtration rate, such as inulin clearance, blood urea nitrogen, and serum creatinine, correlated with survival. The survival probability rate of patients with renal failure 3, 12, and 24 mo after inclusion in the study was 54%, 39%, and 33%, respectively. These figures contrast sharply with those obtained in cirrhotic patients with an inulin clearance >50 ml/min (81%, 61%, and 53%, respectively). As previously reported by our group (11), urinary sodium excretion was also of prognostic value in the series of patients under discussion. Cirrhotic patients with ascites and marked sodium retention showed a poor survival. The renal ability to excrete free water, as estimated by plasma sodium concentration, also correlated with survival. Other parameters of renal function with prognostic significance were urinary potassium excretion and urine osmolality.

Patients with cirrhosis and ascites present a systemic hemodynamic disturbance characterized by arterial hypotension, hypervolemia, high cardiac index, and low peripheral resistance (33,34). Several studies strongly suggest that the cause of these systemic hemodynamic abnormalities is a marked splanchnic arteriolar vasodilation (28,29,35,36). The increased activity of the renin-angiotensin and sympathetic nervous system of patients with cirrhosis with ascites would be a homeostatic response to maintain arterial pressure within or near normal levels (37). Therefore, it is likely that mean arterial pressure, plasma renin activity, and plasma norepinephrine concentration were of prognostic significance in our patients because they reflected the degree of alteration of splanchnic and portal circulation. An alternative hypothesis, however, is that the homeostatic responses caused by the decrease in arterial pressure may have deleterious effects in these patients. The activation of the renin-angiotensin and sympathetic nervous systems may play an important role in the pathogenesis of functional renal failure (6,7) and may also contribute to the altered hepatic hemodynamics present in these patients (37–44).

The multivariate analysis disclosed seven independent predictors of survival. Mean arterial pressure and plasma norepinephrine concentration were the variables that best predicted prognosis. Two other parameters that independently correlated with survival were urinary sodium excretion and glomerular filtration rate. Finally, only one laboratory datum of hepatic function (serum albumin concentration) had independent prognostic value. These findings, therefore, indicate that variables estimating systemic hemodynamics and renal function in cirrhosis with ascites are better predictors of survival than those routinely used to assess hepatic function.

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β-Adrenergic Blockers and Nitrovasodilators for the Treatment of Portal Hypertension: The Good, the Bad, the Ugly

See article on page 1632.

It is already a decade since the publication of the first study showing that pharmacological therapy can effectively ameliorate the incidence of the first variceal hemorrhage in patients with cirrhosis and portal hypertension.[1] Since then several studies have confirmed this initial result.[2] The series of studies comparing nonselective β-adrenergic blockers with placebo for the prevention of the first variceal hemorrhage have changed forever the treatment of cirrhosis and its main, portal-hypertensive complication. It is now widely accepted that patients with cirrhosis and gastroesophageal varices (moderate to large size) should undergo prophylactic pharmacotherapy to prevent the first variceal hemorrhage.[3] Meta-analysis of all the studies published also suggest that this preventive treatment is accompanied by an improvement in survival.

The Good

As we are celebrating the 10th anniversary of this important development in the treatment of liver diseases, it is fitting to publish the long-term results of a randomized trial for the prophylaxis of the first episode of variceal hemorrhage, comparing the effects of the long-acting nitrovasodilator isosorbide-5-mononitrate (ISMN) with the β-adrenergic blocker propranolol. In the first report of this trial, published also in GASTROENTEROLOGY, patients had been followed up for a median of 29 months.[4] In the follow-up report, published in this issue, patients have been followed up for up to 91 months.[5]

Since their introduction,[6] nonselective β-adrenergic blockers have been the drugs of choice for prophylaxis of the first variceal hemorrhage; however, a relatively important proportion of patients with cirrhosis cannot tolerate β-adrenergic blockers or respond poorly to the portal pressure–lowering effect of these compounds.[2,7] Therefore, the 1993 study by Angelico et al.,[4] showing that a long-acting nitrovasodilator ISMN was as effective as the β-adrenergic blocker propranolol in preventing the first variceal hemorrhage, was received with relief, enthusiasm, hope, and also some degree of caution by many of us. Relief, because we had an effective alternative for patients who do not tolerate β-adrenergic blockers. Enthusiasm, because this study confirmed clinically what earlier experimental and hemodynamic studies suggested, i.e., that although the mechanism of action is different,[8] nitrovasodilators are as effective portal-hypotensive agents as β-adrenergic blockers.[9,10] The first report by Angelico et al.,[4] showing safety and efficacy of long-term therapy with a long-acting nitrovasodilator, opened the clinical arena not only for alternative therapy to β-adrenergic blockers but also to combination therapy using these two compounds. The combination of β-blockers and nitrovasodilators increases the portal pressure–lowering effect of either drug used separately.[9,10] This finding is especially hopeful for a group of patients who were unresponsive or poorly responsive to β-adrenergic blockers alone.[7,11]

The Bad

Because it had been known for a long time that patients with advanced cirrhosis are characterized hemodynamically by vasodilatation, it was not without a reasonable amount of concern that we began to treat patients with early cirrhosis, varices, and intolerance to β-blockers with nitrovasodilators, a drug class known to induce vasodilatation with lowering of the arterial pressure. Vasodilatation in liver disease is the hemodynamic expression of liver failure.[12] Over the years we have learned that the clinical expression of vasodilatation, i.e., the level of

arterial pressure, is an excellent prognostic indicator for morbidity and mortality in patients with cirrhosis.[13]

Nitrovasodilators are powerful venous dilators and mild arterial dilators.[14] One of the main indications for this group of drugs, other than treatment of angina, is for the treatment of heart failure. By increasing the capacitance of the venous system, these compounds reduce the venous return to the heart and by that mechanism increase the blood volume that can be accommodated in the venous system.[14] Although this is of tremendous benefit in the treatment of congestive heart failure, this effect is potentially detrimental in patients with cirrhosis. Venous dilation increases the capacitance of the vascular tree and induces a state of relative hypovolemia, a common feature of advanced cirrhosis.[12] Nitrovasodilators may in fact aggravate this state. This detrimental effect of nitrates had already been noticed in earlier studies by Salmeron et al.[15] They showed that a single oral dose of the nitrovasodilator ISMN was accompanied by clear laboratory signs of relative hypovolemia, especially in patients with advanced cirrhosis complicated by ascites. In fact, the hemodynamic and laboratory changes are similar to what recently has been named postparacentesis circulatory dysfunction.[16] This syndrome is characterized by accentuation of clinical and laboratory signs of increasing vasodilatation with relative hypovolemia (activation of the renin-angiotensin and sympathetic nervous system) after a large-volume paracentesis[16] and has been associated with a significant reduction in the probability of survival in comparison to patients who do not respond to paracentesis with signs of relative hypovolemia.[17]

With increasing knowledge about the mechanism by which nitrovasodilators induce vasodilatation and about the pathophysiology of portal hypertension, another important link between nitrovasodilators and cirrhosis has emerged. Nitrovasodilators are compounds that exert their vasodilatory action through the donation of nitric oxide (NO), a naturally occurring gas derived from the essential amino acid L-arginine.[14] NO is a potent endothelial vasodilator whose concentration is known to be increased in chronic liver diseases[18] and is postulated to be the main culprit in the vasodilatation observed in cirrhosis.[12] Therefore, administration of nitrovasodilators, if anything, may accelerate the progression of the vasodilatory syndrome observed in chronic liver diseases with a significant number of resultant potential complications.[12]

However, the news is not all bad. Evidence is mounting to indicate that in cirrhosis, simultaneously with the excess synthesis of NO in the systemic vasculature, there may be a deficit in the synthesis of NO by the endothelial cells of the liver microcirculation.[19] Therefore, the availability of drugs, such as nitrovasodilators, that can replace NO in the liver microcirculatory bed, where NO is deficient, may add a new dimension to the treatment of portal hypertension. Still, it is important to deliver the drug where the drug is needed. Unfortunately, the more advanced the liver disease, the less accessible the liver microcirculation becomes to compounds that are carried by the portal flow and rapidly metabolized when exposed to other vascular beds. Patients with advanced liver diseases have a considerable amount of portal-systemic collateralization that develops as a consequence of portal hypertension. A large fraction of portal blood escapes through these collateral vessels and carries with it compounds that were targeted to the liver. This situation may be one of double jeopardy to the patient with advanced liver disease receiving oral nitrovasodilators because, on one hand, the liver microcirculation is deprived of the beneficial effect of the NO-producing drugs and, on the other hand, the availability of large amounts of NO to the collateral circulation may contribute to further development of this aberrant circulatory bed and an increase in well-known detrimental consequences.[20]

The apparently greater effect of nitrovasodilators in different vascular beds may relate to their bioavailability and differential cellular metabolism of nitrovasodilators to NO. Data seem to indicate that both the liver microcirculation and the collateral circulation are highly responsive to nitrovasodilators.[21,22] Consequently, the potential for a beneficial or detrimental effect of the nitrovasodilators may depend on how advanced the liver disease is and, therefore, how extensively the collateralization of the portal system has developed. Therefore, there is more justification for using nitrovasodilators earlier in cirrhosis, when collateral vessels are poorly developed or not developed and the liver microvasculature will be the main target of the drug.

The Ug y

The paper reported in this issue of GASTROENTEROLOGY by Angelico et al.[5] emphasizes the importance of long-term follow-up of studies that deal with the treatment of chronic diseases that may evolve over many years. In their initial report,[4] they found that ISMN was as effective as propranolol in preventing the first variceal hemorrhage. In fact, patients treated with ISMN had fewer side effects than those treated with propranolol. However, at the end of the observation period, a disturbingly large number of patients had developed liver failure in the group treated with ISMN and mortality increased in this group, especially in patients older than 50 years old. Treatments that may seem to produce short-

term favorable outcomes of symptomatic manifestations of the disease process may in the long run induce a more definitive negative outcome. This effect has been found earlier with other compounds in other areas of medicine. Long-term treatment of portal hypertension with long-acting nitrovasodilators seems to be in this category.

The study by Angelico et al. was originally designed as a single-blinded, 2-year study with a strict follow-up. After the first 2 years, the follow-up became less strict: patients were seen every 4–6 months (sometimes just contacted by phone). Such a reduction in contact between patients and investigators usually results in a diminution of patient compliance and makes the characterization of clinical events more difficult. Additionally, approximately half of the patients in each group withdrew from therapy. For these reasons, I would not like to dismiss nitrovasodilators just yet. I would rather say that this study provides a very serious warning that requires confirmation and a redefinition of what specific circumstances justify the use of nitrovasodilators alone for the treatment of portal hypertension.

Nitrovasodilators were introduced in the treatment of portal hypertension to ameliorate the detrimental effects of the vasoconstrictor vasopressin on the heart and systemic circulation during the treatment of acute variceal hemorrhage.[23] It was noticed that its use in combination was superior to vasopressin alone in ameliorating not only the detrimental effects of vasopressin in the systemic circulation but also in enhancing the portal pressure–lowering effect of vasopressin.[23] Controlled clinical trials have supported the value of this combination.[2] Recently, several studies showed that the combination of long-acting nitrovasodilators and β-adrenergic blocker is superior to the use of β-adrenergic blockers alone in the prevention of variceal bleeding and rebleeding.[24–26] Interestingly, some of the undesirable effects of nitrovasodilators seem to be eliminated by the association of β-blockers with nitrovasodilators.[27] Perhaps the most important protective effect of β-blockers is related to the suppression of signs of relative hypovolemia such as the activation of the renin-angiotensin aldosterone system and the progression of the hyperdynamic syndrome. It therefore seems, at least based on these studies, that combination therapy may be safer because it does not induce the same undesirable effects as have been found with the use of ISMN alone. This observation is reassuring, but it is not a justification for routine use. In my view we should use this combination therapy only when it is really needed, e.g., in patients who are poorly or nonresponsive to therapy with β-adrenergic blockers alone. Patients who respond within 2 or 3 months to the sole use of β-adrenergic blockers with a decrease in the hepatic venous pressure gradient of more than 20% from the baseline value or with a decrease in the hepatic venous pressure gradient to absolute values of less than 12 mm Hg should not receive nitrovasodilators or any other combination therapy. Patients who have had such a significant response to β-blockers have a negligible risk for variceal hemorrhage.[28–30]

Conc usions

In this study, as pointed out by the authors, the reasons for the apparent superiority of propranolol to ISMN are unknown. An unanswered question is whether the difference in survival is caused mainly by the beneficial effect of propranolol or a detrimental effect of ISMN. The truth probably lies in the middle. The evidence seems to indicate that long-term use of ISMN is associated with detrimental complications in patients with chronic liver diseases, but there are many reasons to believe that an increase in survival is another gratifying result of the long-term use of β-adrenergic blockers. β-Adrenergic blockers are clearly the therapy of choice for the prevention of the first variceal hemorrhage.

I think that physicians caring for patients with cirrhosis and gastroesophageal varices (moderate to large size) that have never bled should treat these patients with nonselective β-adrenergic blockers and continue with this therapy indefinitely or as long as the condition of the patient will allow it. The efficacy and safety record of these drugs is reassuring, and the study by Angelico et al. further adds to this record. If the patient is unresponsive to therapy with β-blockers, a long-acting nitrovasodilator should be added to try to optimize the portal-hypotensive effect. The question is what to do with the small number of patients who do not tolerate or have contraindications to the administration of β-adrenergic blockers (~15%–20% of the cirrhotic patients treated with β-blockers). Although nitrovasodilators are probably safe in nonascitic cirrhotics, and perhaps even beneficial in the early stages of the disease, a comparative study against placebo seems appropriate. In high-risk (large varices) cirrhotic patients with ascites who do not tolerate β-blockers, a multicenter controlled clinical trial comparing long-acting nitrovasodilators with endoscopic banding or some other pharmacological approach for the prophylaxis of the first variceal hemorrhage seems justified, although this group of patients may be too small to evaluate in comparative studies. For patients who do not tolerate β-blockers, physicians in consultation with their patients may have to make individual decisions that should be based in the patient's potential risk for variceal hemorrhage and the degree of liver decompensation. No active treatment, long-acting nitrovasodilators, or endo-

scopic therapy may all be appropriate in individual patients.

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